155

MULTIPLEXED GENOTYPING OF HUMAN MINOR HISTOCOMPATIBILITY ANTIGENS

Pietz, B.C.; DuChateau, B.K.; Warden, M.B.; Ellis, T.M. The Blood Center of S.E. Wisconsin, Milwaukee, WI.

Minor histocompatibility antigens (mHA) induce T cell mediated immune responses that have been associated with increased risk of graft versus host disease and allograft rejection. Unlike HLA genes, mHA are encoded by genetically and functionally unrelated genes located throughout the chromosome. The role of mHA in stem cell transplantation and the frequencies of mHA alleles in large study populations remain unknown due in part to the lack of a suitable high throughput method for mHA genotyping. Here we describe the development and utility of a single tube, expandable, multiplexed assay for genotyping mHA (HA-1, HA-2, HA-3, HA-8, HB-1, $CD31^{125}$, and $CD31^{563}$) using the Luminex platform. The assay utilizes a multiplexed allele-independent gated amplification of mHA genes followed by differential detection of allele-specific primer products using the MultiCode system (Eragen Biosciences, Madison, WI). The alleles are interrogated using a multiplex allele-specific primer extension reaction with primers tagged with EraCodes, the products hybridized to Luminex beads, and the hybridization duplex detected using streptavidin-PE. Using this assay, we generated an estimate of mHA allele frequencies in a limited local donor population and are assessing the influence of mHA disparities on outcomes of stem cell transplantation in a large cohort of unrelated recipient-donor pairs.

156

MOLECULAR CHARACTERIZATION OF HLA-A MISMATCHES FOR MODELING OUTCOMES ANALYSES

Baxter-Lowe, L.A.[1]; Maiers, M.[2]; Spellman, S.[2]; Fernandez-Vina, M.[3]; Horowitz, M.[4]; Haagenson, M.[2]; Hurley, C.[5] 1. UCSF, San Francisco, CA; 2. NMDP, Minneapolis, MN; 3. M.D. Anderson, Houston, TX; 4. Medical College of Wisconsin, Milwaukee, WI; 5. Georgetown University, Washington, DC.

For allogeneic transplant candidates who have no HLA matched donor, there are no well accepted guidelines for selecting an HLA mismatched (MM) donor. To provide a foundation for developing new models for ranking HLA MM donors, this investigation focuses on HLA-A MM because single HLA-A MM are observed in a large proportion of patients. The molecular characteristics of 832 HLA-A MM were examined in 3,687 unrelated donor-recipient pairs from NMDP facilitated transplants which had retrospective high resolution HLA typing. There are a few high frequency MM combinations that constitute a large proportion of the MM: 0201 vs 0205 (8%), 0201 vs 0206 (8%), 6801 vs 6802 (4%), 3001 vs 3002 (4%), and 0201 vs 6801 (4%). There are 12 MM combinations that occur $\geq$10 times and comprise 25% of the MM combinations. The remaining MM combinations, which constitute 46% of the total, are relatively infrequent and include 95 combinations that occur only once in this population. Comparison of the protein sequences of the peptide binding domain of mismatched HLAs revealed 1–28 amino acid differences. Of these, 17% were single amino acid differences and 16% had 2–3 amino acid differences. Approximately 70% of MM amino acids were located in positions that alter the environment of the bound peptide. For these residues, small differences in the properties of the amino acid side chains can have substantial effects on T cell allorecognition. Many HLA-A disparities (63%) were accompanied by additional HLA-A, -B, -C, and/or -DRB1 MM. This emphasizes the need for models that detect additive, synergistic, dominant and permissive effects. The characteristics of HLA-A disparities suggest that traditional HLA MM criteria that have been used for outcomes analyses may obscure important molecular characteristics that underlie detrimental or permissive HLA MM. The characteristics of the HLA-A MM are likely to be unique and HLA locus-specific characteristics should be carefully considered in future study designs. A multifaceted approach is proposed for studying the relationship between HLA disparity and transplant outcomes that determines the risks associated with (1) high/intermediate frequency disparities, (2) categories of functionally similar HLA disparities, and (3) multiple HLA MM with complex interactions. This multifaceted approach is likely to provide clinically useful guidelines for selection of MM donors and explain some of the controversies in the scientific literature.

IMMUNE RECONSTITUTION

157

IMPACT OF PLASMACYTOID DENDRITIC CELL RECOVERY ON OUTCOME AFTER REDUCED-INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

Mohty, M.; Blaise, D.; Faucher, C.; Bardou, V.J.; Gastaut, J.A.; Viens, P.; Olive, D.; Gaugler, B. Institut Paoli-Calmettes, Marseille, France.

Among DC subsets, the reconstitution of the natural type I-interferon-producing plasmacytoid DCs (PDC) has been proposed to play a major role in establishing immune competence, given the capacity of PDCs to efficiently expand either specific cytotoxic T lymphocytes, or to promote regulatory T cells, contributing to an impaired immune response. Therefore, we investigated the impact of circulating PDCs measured at 3 months after RIC-allo-SCT, in 54 pts who received a RIC-allo-SCT from an HLA-identical sibling, in order to determine whether this could provide a convenient indicator for long term outcome. The median absolute count of PDCs measured at 3 months was 0.725/μL (range, 0–23.2). In a multiple logistic regression analysis including relevant parameters, only the absence of clinically significant grade II-IV acute GVHD was associated with an improved PDC recovery (P=0.003; OR=6.4; 95% CI, 1.9–22). We also investigated whether PDCs recovered after allo-SCT are functional in response to viral stimulation. Pts experiencing grade 0-I aGVHD could secrete significantly higher amounts of IFN-alpha as compared to pts with grade II-IV aGVHD (mean, 91 pg/ml vs. 0 pg/ml respectively; P=0.002), likely highlighting the deleterious impact of corticosteroids therapy on PDC function. The $CD34^{+}$ stem cell dose and other lymphoid subsets infused with the allograft did not affect PDC recovery. Though PDC count could not predict death from progression or relapse, pts with a high PDC recovery profile had an improved overall survival (OS; P=0.03), in contrast to pts with a low PDC recovery profile who had an increased incidence of late transplant-related mortality (GVHD, infections) (P=0.03). In addition, the overall incidence of late infections (viral, fungal and bacterial) was significantly higher in the low PDC recovery group as compared to the high PDC recovery group (59% vs. 19%; P=0.002), illustrating the importance of PDCs in anti-infectious immune responses. In a multivariate analysis, only a high PDC count was significantly predictive of a decreased risk of death (P=0.04; RR=0.34; 95% CI, 0.12–0.96). The role and impact of rare immune effector cells would tend to be more evident in truly RIC and less toxic regimens. In this study, we could show that monitoring of PDCs may be useful for pts management (closer surveillance, infection prophylaxis), and may have a significant impact on the probability of a favorable outcome in the context of RIC-allo-SCT.

158

INTERLEUKIN-7 IMPROVES RECONSTITUTION OF ANTIVIRAL CD4 T CELLS

Lu, H.; Zhao, Z.; Kalina, T.; Kiem, H.-P.; Storek, J. Fred Hutchinson Cancer Research Center, Seattle, WA.

We evaluated whether long-term (2 months) administration of interleukin-7 (IL7) hastens immune recovery in baboons rendered severely lymphopenic by total body irradiation and anti-thymocyte globulin. Four baboons were treated with recombinant baboon IL7 and three baboons with placebo. Median CD4 T cell count at the end of IL7/placebo treatment was higher in the IL7-treated animals (2,262 vs 618/ml, p=0.03). This appeared to be a result of peripheral expansion rather than de novo generation. Median cytomegalovirus-specific IFNg-producing CD4 T cell count at the